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Insecticidal Activity and Chemical Composition of *Moringa oleifera* Extract Against The Leguminous Aphid, *Aphis craccivora* Koch on Broad Bean Plants

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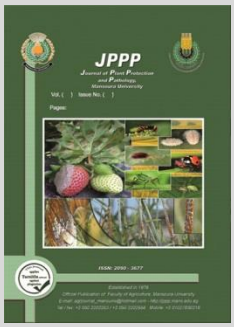

ABSTRACT

In this study, *Moringa oleifera* leaves were extracted by ethanol 70% and the contents of total phenolic and total flavonoids were estimated by using spectrophotometer methods and HPLC analysis. The content of total phenolic in the ethanolic extract of *M. oleifera* leaves was 11.66 g GAE/100 g ethanolic extract. The content of total flavonoids in the ethanolic extract of *M. oleifera* leaves was 4.52 g IQE/100g extract. Twenty five components were represented in the tested sample by HPLC. The study also aimed to evaluate the *M. oleifera* extract (ethanolic extract) prepared from *M. oleifera* leaves, as a natural insecticide and thiamethoxam (80 g / fed.) as a comparative insecticide against aphid, *Aphis craccivora* on broad bean (*Vicia faba* L.) plants in Faculty of Agriculture farm, Sharkia Governorate, Egypt during the 2017 season. A series of concentrations were prepared from *M. oleifera* extract, concentrations 1, 2, 4 and 8%. The results obtained that the reduction percentage in the population of aphid insect increases with increasing concentration of *M. oleifera* extract (1% - 8%). The concentrations of 2 and 4% of *M. oleifera* extract caused 48.66 and 58.95 % mortality, respectively. The results also revealed that there is no significant differences between thiamethoxam and 8% *M. oleifera* leaf extract, there are recorded the highest values in reducing the population of the insect tested compared with the control treatment. The results indicate that biomaterials derived from *M. oleifera* leaves can be used for integrated pest management, which is a good alternative to conventional synthetic insecticides.

Keywords: *Moringa oleifera*, ethanolic extract, HPLC analysis, bioactive compounds, efficiency, *Aphis craccivora*, thiamethoxam.

INTRODUCTION

Broad bean (*Vicia faba* L.) plants are a legume crop grown primarily for its edible seeds (beans). Broad bean is a major legume seed consumed by humans and animals worldwide. *A. craccivora* (Koch) colonizes infested the shoots of many plants, in particular crops of Leguminosae (Blackman and Eastop, 2006). Aphids are small, soft-bodied insects and its feed on more than 50 plant families (Blackman and Eastop, 2000).Furthermore, this aphid is involved in the transmission of about 30 persistent and semi-persistent phytoviruses (Blackman and Eastop, 2007).

Besides sucking the sap, *A. craccivora* (Koch), also secrets sugar rich honeydew which causes black sooty mould on the leaves. Protection of plants from the insect damage is recently dependent on synthetic pesticides. The repeated apply of synthetic insecticides for controlling the pests has disrupted natural biological control systems. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concerns, which initiated a search for alternative control measures (Adeniyi *et al*., 2010). Now, there are a number of bio-pesticide plant extracts being marketed as insecticides, it is used as insecticides are fast acting, quickly decreasing insect feeding and crop damage (John and Jimmy, 2015). Moringa, considered as one of the world's most useful trees (Ojiako *et al*., 2012). *Moringa Oleifera* belongs to the family Moringaceae which possess additional 13 species (Keay, 1989). According to Fugile (2000) the many uses for *M. Oleifera* include: fertilizer, biopesticide, medicine etc. Botanical pesticides are namely, bio-pesticides and it is important alternatives to reduce or replace the use of the synthetic pesticides (Halder *et al*., 2007 and Dougoud *et al*., 2019). It is also, not available and may be expensive (Amoabeng *et al*., 2014 and Dougoud *et al*., 2018). Allelochemics affect in the behavior or population biology of insects (Berlinger, 2008). Phenols are the main group of minor metabolites (Lattanzio *et al*., 2006). Thiamethoxam is a neonicotinoids act on nicotinic acetylcholine receptor in the pests treated, preventing their supplying reflex and 3-[(2-chloro-5-thiazolyl) methyl] tetrahydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine .

Due to the problems of conventional pesticides, there is need to develop complementary and/or alternative cheap but effective and safe anti-aphid plant-derived biopesticide. The main objective of our study the effect of ethanolic extract of *M. oleifera* plant on *A. craccivora* found on broad bean plants under field conditions.

MATERIALS AND METHODS

Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS),

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gallic acid and quercetin were obtained from Sigma (St. Louis, MO, USA). Ethanol was purchased from El-Nasr Company for Chemical Industries, Cairo, Egypt. All other chemicals used in experiments were of analytical grade.

Materials

Leaves of *M. oleifera*, was collected from the Herbarium of Orman Botanical Garden, Ministry of Agriculture, Giza, Egypt.

Preparation of defatted moringa leaves flour

Moringa leaves were manually cleaned and ground for 3 min using a Moulinex mixer (Type 716, France) at the maximum speed setting and the meals were ground to pass through a 1 mm^2 sieve. The powder was then defatted using *n*-hexane and the fat content was determined by A.O.A.C. (2000). Solvent was discarded by rotary-evaporator and dehydrated-defatted flour was stocked at 4 °C.

Preparation of the extracts

Twenty gram of sample was extracted with 200 ml ethanol 70% using magnetic stirrer for 2h at room temperature followed by filtration through Whatman No.1 filter paper. The remnants were re-removed under the same conditions, then ethanol 70% extracts were freeze- dried (Thermo- electron Corporation –Freeze dryer). The dried extract after evaporation of solvent was stored at -20°C until tests executed.

Total phenolic content

Total phenolic compounds (TPC)

The TPC in all extracts (1000 µg/ml) were measured at O.D 760 nm according to the method described by Kahkonen *et al.* (1999) using UV spectrophotometer (Jenway-6405-UV/VIS). TPC content expressed as gallic acid equivalent (GAE) were calculated based on the calibration curve using the following linear equation:

$$Y = 0.001X + 0.0563 \qquad R2 = 0.9792$$

X= Concentration (ppm)

Phenolic compounds analysis by HPLC

Phenolic compounds were estimated by HPLC according to the method of Goupy *et al*., (1999)

Total Flavonoids

Total flavonoids content (TF)

The TF content in extract (1000 µg/ml) were measured at O.D 420 nm according to the method of Ordon *et al*. (2006) using UV spectrophotometer (Jenway-6405-UV/VIS). Total flavonoids content expressed as quercetin equivalent (QE) was calculated based on the calibration curve using the following equation:

$$Y = 0.0012x + 0.008 \qquad R2 = 0.944$$

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Flavonoids compounds analysis by HPLC

Flavonoid compounds of extract were determined by HPLC according to the method of Mattila *et al.* (2000).

Efficacy of extract of *Moringa oleifera* and thiamethoxam against the *Aphis craccivora* Koch

Field Experiments design

Field experiments were conducted to evaluate the efficacy of curde extract of *M. oleifera* against the *A. craccivora* **Koch** (Hemiptera: Aphididae), infesting broad bean plants. The experiment included seven treatments were carried out at Faculty of Agriculture Farm, Sharkia Governorate, Egypt during 2017season. The treatments were carried out in randomized complete block design with three replicates for each treatment. The tested extract was tested in three concentrations beside thiamethoxam (Simo 25 % WG) [R] at rate 80 g / fed. was used as standard insecticide. For control no chemical was applied on the broad bean plants, only water was applied. Tween 80 at 0.05% was used as an emulsifier agent alone and in combination with the final test solutions of extract. From the stock solution of plant extract, test solutions were prepared in the concentrations of 1, 2, 4 and 8 %. Spraying method was used to evaluate the insecticidal activity using a knapsack hand sprayer fitted with one nozzle bloom (200 L./fed.) was used. Investigated compounds were applied twice at15 days interval between sprays. The first spray was sprayed on November 10th. The tested insects were recorded before spraying and 3, 7 and 10 days after treatment. Initial effect recorded in one day after spray, the residual effect during the period from 3rd till 14th day after spray. Sample of 75 leaves from 75 plants were randomly collected from each treatment. Field samples of broad bean leaves treated were collected and placed in paper bags, brought immediately to the laboratories of Plant Protection Department, Faculty of Agriculture, Zagazig Univ., Sharkia. The moving stages (immature and adults) aphid in the tested crop which treated with different compounds were counted and mortality percentages were calculated under stereoscopic binocular microscope. All the tested stages responding to touching with camels hair brush were consider alive one. Statistical analyses to compare the efficiency of the treatments in controlling the mite, the daily number of the tested stages individuals alive were analyzed using ANOVA with Duncan (1955) test at the 95% confidence level. The percentage reduction in population of the moving stages (immature and adults) aphid in the tested crop was calculated according to Henderson and Tilton's (1955) formula as follows:

$$R = 100 \cdot 1 - (Ta \cdot Cb) / Tb \cdot Ca)$$

Where:

R = Reduction percentage in infestation
C = Insect number in the untreated control plot.
T = Insect number in the treated plot.
a = After insecticide application.
b = Before insecticide application.

RESULTS AND DISCUSSION

Total phenolic and total flavonoids content

The contents of total phenolic and total flavonoids of *M. oleifera* leaves using ethanol 70% are listed in Figure 1.

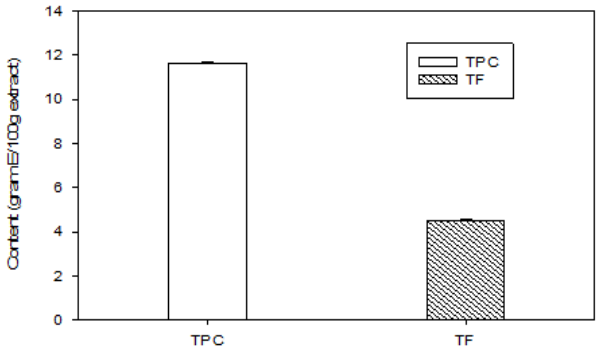

Figure 1. The content of total phenolic and total flavonoids of *M. oleifera* leaves using ethanol 70%.

TPC=Total Phenolic Compounds TF=Total Flavonoids

The content of total phenolic in the ethanolic extract of *M.oleifera* leaves was 11.66 as g gallic acid equivantent /100 g ethanolic extract. The content of total flavonoids in the ethanolic extract of *M. oleifera* leaves was 4.52 as g guercetin equivantent /100 g ethanolic extract. Higher levels of total phenolics and total flavonoids in *M. oleifera* leaves were observed by Sreelatha and Padma (2009).Vongsak *et al.* (2013) recorded that soaking with ethyl alcohol (70%) of dried leaves confirmed the extract with maximum amounts of total flavonoids and total phenolics.

The phenolic compounds identified in *M. oleifera* ethanolic extract are listed in Table (1). Twenty five components were represented in the tested sample. The main components were 3-OH-tyrosol, chlorogenic and catechol (1339.29, 317.29 and 274.29 ppm, respectively).The flavonoid compounds identified in *M. oleifera* ethanolic extract are listed in are listed in Table (2).

Table 1. Bioactive phenolic compounds in *Moringa oleifera*, leaves 70% ethanolic extract evaluated by HPLC.

phenolic compounds (ppm)	Concentration (ppm)
Gallic	5.36
Pyrogallol	234.36
4-Amino benzoic	5.71
3-OH Tyrosol	1339.29
Protocatchuic	10.76
Catehein	37.64
Chlorogenic	317.29
Catechol	274.29
Epicatechein	24.23
Caffiene	8.13
P-OH.benzoic	69.18
Caffeic	5.89
Vanillic	26.47
Ferulic	14.67
Iso-ferulic	9.32
Reversetrol	120.16
Ellagic	31.99
E-vanillic	261.01
Alpha-coumaric	7.52
Benzoic	184.14
3,4,5-methoxy-cinnamic	7.37
Coumarin	0.00
Salicylic	76.36
P-coumaric	12.01
Cinnamic	37.77

Table 2. Bioactive flavonoid compounds in in *Moringa oleifera* leaves 70% ethanolic extract evaluated by HPLC.

Flavonoids (ppm)	Concentration (ppm)
Naringin	53.61
Rutin	340.79
Hisperdin	3846.97
Rosmarinic	53.39
Quercetrin	66.44
Quercetin	10.06
Narengenin	2.57
Kampherol	5.61
Hispertin	11.35
Apegenin	3.92
7-OH flavone	3.37

Eleven components were represented in the tested sample. The main components for *M. oleifera* were hisperdin and rutin (3846.97 and 340.79 ppm, respectively).The major active compounds are chlorogenic and isoquercetin (Vongsak *et al.*, 2013).

Insecticidal activity extract of *Moringa oleifera* and thiamethoxam against the *Aphis craccivora* (Koch)

Mortality percentage in individuals of *A.craccivora* (Homoptera: Aphididae) attacking broad bean plants treated with *M. oleifera* extract differed significantly compared to other treatments ($P \leq 0.05$). Aphids in this treatment suffered the highest mortality with 78.03 %. In fact, the number of *A. craccivora* differed significantly for all treatments less than the untreated control except tween-80. Moringa plant extracts were able to control the targeted aphid. At 1 % of *M. oleifera* extract was recorded to be 34.13 % as general mean against the *A. craccivora* compared with control (Table 3). The concentration of 2% of *M. oleifera* extract caused 48.66 % mortality while, the concentration of 4 % *M. oleifera* gave 58.95 percent mortality as general mean in 2017 season. At 8 % concentration 78.03 % *A. craccivora* were died when treated with *M. oleifera* extract. Mortality percentage was nil in control condition. Percent mortality increases with increasing the concentrations (1 % and 8 %) for *A. craccivora* .However, no significant difference was observed between thiamethoxam at 80 g/ fed. and methanolic extract at the concentration of 8 %. According to the reduction percentages, thiamethoxam (80 g/fed.) also gave better toxicity against *A. craccivora*. However, field evaluation of ethanol extract from *M. oleifera* leaves (1-8%) caused decreasing in counting *A. craccivora* and showed significant insecticidal effect as compared with untreated plot. However, tween- 80 alone at 0.05% was nontoxic compared to the control and recorded the lowest significant values. The *A. craccivora* (Koch) is polyphagous pest causing economic damage in several agricultural crops throughout the world. In addition, huge use of insecticides resulted in the development of resistant strains of aphids (Harmel *et al*., 2008). As a result, world have start to look for other ways to dominance these insects (Jordan, 2013). The plant scholar is a very important exporter of bio-pesticide compounds and also supply substance structures from which new and more efficient pest control agents can be advanced. In these trials novel types of insecticide producing from natural products, targeting the *A. craccivora* Koch could be helpful stand by for integrated pest management. Most plant kinds that are used in phytomedicine contain ingredients, which block the growth of the insects, prevent their feeding (antifeedants) or act as repellents and confessants (Laznik *et al*., 2010).In another study, to decrease the use of pesticides on crop plantations, by using bio-pesticides with minimize health and environmental effect (Dancewicz *et al.*, 2011).

However, *M. oleifera* has been cultivated and introduced in Egypt. Methanolic extracts of aqueous extract of moringa seeds exhibited larvicidal action against *Aedes aegypti* (Kamara and Rahuman (2010). The author Aioub *et al.*, (2015) had stated that thiamethoxam at 1/2 and 1 recommended dose is extremely toxic to *A. craccivora* under field conditions. Similar reports of insecticidal activity were observed also with Lebbal *et al.*,

(2017) test the effect of 11 aqueous plant extracts on the aphid *Aphis craccivora*. They added that the ethanolic extracts were efficacious against *A. craccivora*. Prabhu *et al.* (2011) had recorded that the phytochemicals derived from *M. oleifera* seeds extracts are effective mosquito vector control agents. Also, Ojiako *et al.*, (2013) had found that *M. oleifera* seed extracts reduced the number of *Megalurothrips sjostedti* and *A crassivora*. Also, *M. oleifera* extracts had 62 % reduction of *Phyllotreta cruciferae* (Alao and Adebayo, 2015). Using plant extracts as bio-pesticides offering the farmers many unique benefits, it is usually are inherently less harmful than conventional the pesticides (John and Jimmy, 2015). Botanical extracts were used for control of the agricultural pests in Egypt since 2006 and decreasing losses in the food production (Dougoud *et al*., 2019).Because the label formulation is easy to obtain and prepare and could be an alternative bio-pesticide for the control of insect pests. Our results suggest that, farmers could apply these bio insecticides first in the field to reduce infestation and these products are readily available, bio-degradable and environmentally friendly. Therefore, could be used in integrated pest management, which are a good alternative to conventional synthetic insecticides.

Table 3. The mean number and reduction percentages of the *Aphis craccivora* (Koch) on broad bean (*Vicia faba* L.) plants in 2017 season.

Treatment		First spray					Second spray					General Mean
		Pre-Count	Days post treatment			Mean	Pre-Count	Days post treatment			Mean	
			3 days	7 days	10 days			3 days	7 days	10 days		
Extract at 1 %	No.	15.46	13.24	9.24	8.32	10.26^{C}	16.32	12.30	8.52	10.25	10.35^{C}	10.30^{C}
	%Red.		(16.84)	(39.07)	(31.99)	(29.30)		(31.92)	(43.95)	(41.03)	(38.96)	(34.13)
Extract at 2 %	No.	16.81	12.58	8.16	6.89	9.20^{BC}	16.80	11.72	6.77	4.96	7.81^{BC}	8.50^{BC}
	% Red		(27.33)	(50.51)	(48.21)	(42.01)		(36.96)	(56.73)	(72.28)	(55.32)	(48.66)
Extract at 4 %	No.	16.90	10.45	7.60	4.84	7.63^{B}	16.28	10.28	5.98	1.80	6.02^{B}	6.82^{B}
	% Red		(39.96)	(54.16)	(63.81)	(52.64)		(42.96)	(63.20)	(89.62)	(65.26)	(58.95)
Extract at 8 %	No.	17.68	9.00	3.09	0.78	4.29^{A}	17.54	9.08	1.74	0.29	3.70^{A}	3.99^{A}
	% Red		(50.57)	(82.18)	(94.42)	(75.72)		(53.24)	(89.35)	(98.45)	(80.34)	(78.03)
Thiamethoxam	No.	16.40	9.25	2.69	0.0	3.98^{A}	15.24	7.84	0.77	0.00	2.87^{A}	3.42^{A}
80 g/ fed.	% Red		(45.23)	(83.28)	(100.00)	(76.17)		(53.53)	(94.58)	(100.00)	(82.70)	(79.43)
Tween 80	No.	23.10	23.79	22.66	18.28	21.57^{D}	19.24	21.30	18.64	20.49	20.14^{D}	20.85^{D}
0.05 %	% Red		(0.00)	(0.00)	(0.00)	(0.00)		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Control		23.10	23.79	22.66	18.28	21.57	19.24	21.30	18.64	20.49	2014	20.85

Means with the same letter in each column are not significant different (p<0.05).
LSD for Treatments =1.5616 LSD for Treatments =1.2973
LSD for Periods =1.1804 LSD for Periods =0.9807

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الكفاءة الإبادية والتركيب الكيميائى لمستخلص *Moringa oleifera* ضد حشرة من البقوليات *Aphis craccivora* Koch على نباتات الفول

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في الدراسة الحالية ، تم استخلاص أوراق المورينجا أوليفيرا بواسطه الإيثانول 70% ، وتم تقدير محتويات مجموع الفينولات والفلافونويدات الكلية باستخدام طرق الطيف وتحليل HPLC. كان محتوى الفينول الكلي في مستخلص الإيثانول من أوراق المورينجا أوليفيرا 11.66 جم حمض الجاليك / 100 جرام مستخلص ايثانولى. كان محتوى الفلافونويدات الكلية في مستخلص الإيثانول لأوراق المورينجا أوليفيرا 4.52 جرام كيرستين / 100 جرام عينه. تم تقدير خمسة وعشرون مركب فينولى في العينة التي تم اختبارها بواسطة HPLC. كما هدفت الدراسة إلى تقييم مستخلص المورينجا أوليفيرا (مستخلص ايثانولى) المحضر من أوراق المورينجا أوليفيرا كمبيد حشري طبيعي ومبيد ثياميثوكسام (80 جم / فدان) كمبيد حشري للمقارنة ضد حشرة المن *A. craccivora* على نباتات الفول (*Vicia faba* L.) تحت الظروف الحقلية في مزرعة كلية الزراعة ، محافظة الشرقية ، مصر خلال موسم 2017. حيث تم تحضير سلسلة من التركيزات من مستخلص المورينجا أوليفيرا، بتركيزات 1 , 2 , 4 و 8 ٪. وأوضحت النتائج أن النسبة المئوية للخفض فى تعداد حشرة المن تزداد مع زيادة تركيز مستخلص أوراق المورينجا أوليفيرا (1٪ إلى 8٪). أحدثت تركيزات 2 و 4 % من مستخلص *M. oleifera* خفض بنسبة 48.66 و 58.95 على التوالي. أظهرت النتائج ايضا عدم وجود فروق معنوية بين مبيد ثياميثوكسام ومستخلص أوراق المورينجا أوليفيرا بتركيز 8٪, حيث سجلا أعلى القيم فى خفض تعداد الحشرة المختبرة بالمقارنة بالكنترول. وتشير النتائج المتحصل عليها إلى امكانية أستخدام المركبات الحيوية المستخلصة من أوراق المورينجا أوليفيرا لاستخدامها في الإدارة المتكاملة للآفات، والتي تعد بديلاً جيدًا للمبيدات الحشرية المصنعة التقليدية.